

## **Gene Chip® Human Exon ST Array (Affymetrix) Neuroblastoma (NBL) - Gene Expression**

RNA was extracted from Optimal Cutting Temperature (OCT) embedded primary tumor tissues using TRIZOL based methods with QIAGEN RNAeasy clean up at either Children's Hospital Los Angeles, Children's Hospital of Philadelphia or the Children's Oncology Group Biopathology Center at Columbus, Ohio.

Manufacturer's protocol was used to label extract, hybridize, and scan the human exon arrays (Affymetrix Human Exon Array Labeled Extract, Affymetrix Human Exon Array Hybridization Protocol, Affymetrix Human Exon Array Scan Protocol).

Level 2 data from normalization and summarization using RMA-sketch analysis of Affymetrix APT tools (version 1.16.0). Level 2 batch effect corrected (BER) data were obtained by removing the batch effect observed related to RNA source of the specimens. Generalized linear model (GLM - R version 3.10) was used to remove institutional batch effect by fitting a model for each of the Human Exon array probeset regions (PSR) to the batch effect (RNA source by institution). This GLM model was adjusted for risk groups based on stage and MYCN amplification status. This Level 2 data was used to generate all subsequent data transformations.

Level 3 based on PSRs that are part of the 'core' annotation. The data was derived from Level 2 BER data. First PSRs with low expression (less than median expression level of entire dataset) and low coefficient of variation (less than median cv of entire dataset) were removed (~10% of PSRs) prior to averaging of PSRs by Transcript ID (based on Affymetrix Annotation). Level 3 based on PSRs that are part of the 'extended' annotation. The data was derived from Level 2 BER data. First PSRs with low expression (less than median expression level of entire dataset) and low coefficient of variation (less than median cv of entire dataset) were removed (~10% of PSRs) prior to averaging of PSRs by Transcript ID (based on Affymetrix Annotation). Level 3 based on PSRs that are part of the 'full' annotation. The data was derived from Level 2 BER data. First PSRs with low expression (less than median expression level of entire dataset) and low coefficient of variation (less than median cv of entire dataset) were removed (~10% of PSRs) prior to averaging of PSRs by Transcript ID (based on Affymetrix Annotation). Level 3 based on PSRs that are part of the 'core' annotation. The data was derived from Level 3 BER transcript data set where PSRs with low expression (less than median expression level of entire dataset) and low coefficient of variation (less than median cv of entire dataset) were removed (~10% of PSRs) prior to averaging of PSRs by Gene Symbol (based on BioCore Package Affymetrix huex10 annotation data - huex10stprobeset.db. Mappings were based on data provided by: Entrez Gene <ftp://ftp.ncbi.nlm.nih.gov/gene/DATA>, with a date stamp from the source of: 2014-Mar13).

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